ESRF

Experiment title:

Time resolved crystallographic studies of an ATP-dependent carboxylase: dethiobiotin synthetase

Experiment number:

LS 320

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Report:

One of the main tasks while doing time resolved experiments is to find a feasible way of starting the reaction in all molecules in a crystal simultaneously. We have explored the possibilities to use diffusion (l), caged substrate that can be activated by laser pulses (2) and pH jump (3) as ways to achieve this. The main part of the experiments (1-3) was done with amonochromatic beam. Some data sets were also collected using a polychromatic beam(4) to investigate the possibilities of the LAUE method. All data was collected using the CCD detector at BL3,1D9 to a resolution of 1.8 -2.0 Å the limiting factor being the size of the detector rather than the diffraction power of the crystals. The monochromatic data sets were of excellent quality with typical R_{merge} of 4 %.

1. In the first experiment we diffused ATP into crystals to start the reaction. Crystals presoaked with the DAPA-substrate were transferred to an ATP containing solution and subsequently flash frozen at 100 K. The soaking time spanned from 95 seconds to 80 minutes. It needed more than 15 minutes of soaking before the nucleotide was clearly present in the electron density map. This indicates that ATP binding to crystalline DTBS is a slow process.

The data set collected after 20 minutes of soaking showed that the y-phosphate of the ATP molecule had been cleaved off, indicating that at least the first step of the reaction had already taken place. There is clear density for the DAPA-substrate while the electron density for the carbon dioxide, bound as carbamate, is weak. It remains to be determined whether the electron density in these crystals corresponds to the enzyme-intermediate complex or just the enzyme-substrate-ADP complex.

In summary, our results suggest that in the crystal, binding of the nucleotide is the time limiting step, between 15-20 minutes, and that the time scale of the actual reaction might be shorter. Initiation of the reaction by soaking the crystal in ATP is therefore not a suitable method in this case.

2. Another way of triggering an ATP dependent reaction is by using caged ATP, a non hydrolyzable ATP analogue. Caged ATP is protected by a dinitrophenyl group that can be cleaved off by photolysis using light with a wavelength of approximately 350 nm. This compound was soaked into the crystals together with substrate. Crystals were exposed to laser pulses of appropriate wavelength and frozen after various time intervals. Unfortunately, during our first visit at ESRF the laser pulses were not of sufficient energy for cleavage of the caged ATP. Therefore no conclusions could be drawn from this experiment.

During a second visit to ESRF (June 96) this experiment was repeated. This time the laser pulse was considerably stronger (4-5 mJ/pulse) and carefully focused on the crystal. The energy input, which should be enough to activate ATP, did not affect the quality of the data considerably. This can be considered as a positive result, but further conclusions must await the evaluation of this data, which is underway.

3. A third way of inducing the activity, by pH jump, was also used during our last visit. This data have not yet been fully evaluated and the results will be enclosed in our next report

4. Attempts to use the advantages of fast data collection at room temperature given by the LAUE method was explored. LAUE images of the native enzyme and enzyme-substrate complexes indicated that this method might be used for DTBS. However laser flashes induce a slight crystal disorder in the enzyme-caged ATP-DAPA complex. This increase in mosaicity is at present an obstacle in using the LAUE method in this project. Therefore we are at date working with monochromatic radiation for data collection. Efforts will be made in the future to evaluate, in more detail, the possibilities for the LAUE method in our project.